Phytochemical Analysis and Anti-Mycobacterial Activity of Some Selected Medicinal Plants

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Abstract: **The research was designed to screen some selected medicinal plants for in-vitro anti-mycobacterial activity. Nine (9) plants (*Erythrina senegalensis, Striga hermonthica, Tamarindus indica, Ximenia Americana, Butyrospermum paradoxum, Euphorbia hirta, Pilostigma reticulatum, Waltheria indica, Cissampelos mucronata*) were used in the study. Extraction was done on Leaf extract of *Waltheria indica*, Stem bark extract of *Ximenia Americana*, Leaf extract of *Pilostigma reticulatum*, Aerial part extract of *Striga hermonthica*, Leaf extract of *Butyrospermum paradoxum*, Root extract of *Cissampelos mucronata*, Whole plant extract of *Euphorbia hirta*, Root-bark extract of *Tamarindus indica* and Stem-bark extract of *Erythrina senegalensis.* The percentage yield of the extraction was 8.2, 9.5, 7.6, 9.4, 10.5, 10.3, 11.6, 9.2 and 8.5 respectively. Preliminary phytochemical analysis on the crude extracts have revealed the presence of the following bioactive chemical constituents; Alkaloids, steroids, terpenoids, flavonoids, Anthraquinones, Saponins, Glycosides, Tannins and Phenols. Preliminary anti-mycobacterial screening was done on the crude plants extracts. The result revealed that only four (4) plants out of the nine (9) selected plants exhibited anti-mycobacterial activity (at conc/well of 500 -125mg/ml) when tested against *Mycobaterium smegmatis* and *Mycobacterium tuberculosis* (Table 3). The crude extract of the aerial parts of *S. hermonthica* inhibited growth of the tested organisms at 500mg/ml but at lower concentrations (125mg/ml) the extract was not active against *M.tuberculosis*. The crude extract of stem bark of *E. senegalensis* was also active at 500mg/ml against the tested organisms but did not demonstrated activity against *M. tuberculosis* at lower concentrations. The root of extract of *C. mucronata* was only active at 500mg/ml but was inactive at lower concentrations and the whole plant extract of *E. hirta* was only active at 500-250mg/ml against both *M.smegmatis* and *M. tuberculosis*. No activity was observed for the other five (5) plants extract used in the study that is *W. indica, X. americana, P. reticulatum, B. paradoxa* and *T. indica.***

Keywords: **phytochemical, medicinal plant, anti-mycobacterial activity**

I. INTRODUCTION

Tuberculosis is caused by *mycobacterium tuberculosis.* Tuberculosis is a common infectious disease caused by various strains of mycobacteria, usually *mycobacterium tuberculosis* (*M. tuberculosis*) (Kumar *et al.*, 2007). The *mycobacterium tuberculosis* usually attacks the lungs and can also attack other parts of the body like kidney, spine and brain. *M. tuberculosis* is spread by air that contains droplets generated by cough, sneeze or spits of a person infected with the bacilli (Konstantinos, 2010). The extracts that inhibited the growth of the mycobacterial pathogens was regarded as possessing anti-tuberculosis activity. The relatively lower toxicities of plant–based medicines as compare to modern pharmaceuticals, coupled with their reduced cost has justified the use of herbal medicines as alternatives for synthetic drugs (Koehn and Carter, 2005). There is increase emergence of drug-resistant TB strains (MDR-TB, XDR-TB) on the currently used anti tuberculosis drugs and lengthy course of treatment of tuberculosis. The Multidrug-Resistant TB (MDR-TB) drugs used are toxic and expensive, while extensively drug Resistant(XDR-TB) tuberculosis is virtually not responding to the currently available drugs (Brennan and Nikaido, 1995). There are number of medicinal plants which posses' significant anti-tuberculosis activities, but their efficacy and safety are not yet assessed and verified.

There are widespread claims by herbalists in the local communities that tuberculosis could be treated with the selected medicinal plants used in the study. But their claims were not supported by scientific evidence. This is because like many African countries, Nigeria has very scanty published literatures on plants screened for anti-mycobacterial activity. The situation is further compounded by the unprecedented loss of biodiversity within the country (Ogundaini, 2005). The results of this study will however authenticate the claims made by the traditional medicine practitioners (TMPs) and will identify plants with anti-mycobacterial activities that could be used in anti-TB drug discovery.

II. METHODOLOGY

Sample Collection and Identification

The plants samples were collected from Three (3) Local Government Areas in Bauchi State, Nigeria using the standard method. The freshly collected plant samples was conveyed in clean polythene bags to Biology Department, ATBU, Bauchi for identification by a taxonomist and voucher specimens was prepared and deposited in the Departmental Herbarium (Abdalfatah, *et al*., 2013; Zailani, *et al*.,2010; Mann, *et al*, 2008; Kubmarawa, *et al*.,2007).

Drying and Pulverising

The samples of different parts of the plants were dried under shade inside a room. The well dried samples were pulverized to fine powder using a wooden mortar and pestle. The powder of each sample was weighed using analytical balance and kept at room temperature (Ibrahim, *et al*., 2012).

Extraction of plant samples

The plants samples were subjected to exhaustive extraction using soxhlet extractor. One hundred grams (100g) of each sample in 70% Methanol was used in the extraction process that lasted for 12hrs. The crude extract of each sample was filtered and concentrated in an oven at 40°c. The dried methanol extracts were then packed in glass bottles and properly labeled.

Phytochemical analysis

The Qualitative analysis of the extracts was carried out in accordance with standard method as described by Harborne (1973), Trease and Evans (1989), Sofowora (1993), (Ghambal, *et al*.,2014). The result of the preliminary qualitative analysis of the plant samples was presented in table 1.

Qualitative Analysis

Test for Alkaloid

1ml of Picric acid solution was added to 2 ml of the extract and observed for orange colouration which indicates the presence of alkaloids (Harbone, 1973),(Trease and Evans, 1989).

Test for Flavonoid

Sodium hydroxide test:

5g of the sample was detanned with acetone. Warmed on water bath to remove acetone. Water was added, warmed and Filtered. 5ml of 10% sodium hydroxide was added to the filterate. A yellow solution indicates the presence of Flavonoids(Harbone,1973), (Trease and Evans,1989).

Test for Terpenoid :

0.2g of extracts was mixed with 2ml Chloroform and 3ml of concentrated sulphuric acid was added carefully to form a layer. A reddish brown coloration at the interface indicates the presence of terpenoids (Sofowara, 1993).

Test for Saponin

Froth test: 10ml of sterile distilled water was added to 1g of the sample and boil for 5minutes. Filtered and 10ml of sterile distilled water was added to the filtrate in a test tube. The test tube was shaken vigorously for about 30seconds and allowed to stand for half an hour. Honey comb froth was observed which indicates the presence of Saponins(Trease and Evans, 1989), (Ghambal *et al*, 2014).

Test for Steroid

2ml of acetic anhydride was added to 0.5g of the extracts followed by 2ml of Sulphuri acid. A violet colour which turned blue was observed which indicates the presence of steroids (Harbone, 1973),(Trease and Evans,1989).

Test for Anthraquinone

0.5g 0f the plant extract was shaken with 10ml of aqueous sulphuric acid and then filtered while hot, the filtrate was then shaken with 5ml of benzene, the benzene layer separated and half its own volume of 10% ammonia solution was then added. A violet or red colouration in the ammonical (lower) phase was observed which indicates the presence of combined Anthraquinones (Sofowara, 1993, Ghambal *et al*.,2014).

Test for Phenol

2ml of ferric chloride solution was added to 25 ml of extract. Deep bluish green solution was formed which indicates the presence of phenol (Trease and Evans, 1989).

Test for Glycosides

25 ml of 1ml Sulphuric acid was added to 5ml of the extract in a test tube and boiled for 15minutes. It was Cooled and neutralized with 10% sodium hydroxide, and then followed by 5ml of fehling solution A and B. A brick red precipitate was observed due to reducing sugars. This indicates the presence of Glycosides (Trease and Evans, 1989), (Ghambal, *et al*.,2014).

Test for Tannins

50ml distilled water was added to 2g of sample and heat for 30mins. Filtered and the portion of the filtrate was diluted with sterile water in a ratio of 1:4. Three (3) drops of 10% ferric chloride solution was then added. A blue or green colour was observed which indicates the presence of tannins (Trease and Evans, 1989).

Preliminary Screening of Samples for Anti-Mycobacterial Activity

After preliminary extraction of the plant samples, the extracts were screened for anti-mycobacterial activity using well diffusion method.

Extract and Organism Preparation

Five hundred milligram (500mg) of each extract was dissolved in 1ml Dimethylsulphoxide (DMSO), centrifuged at 13,000xg for 15minutes. Weighing of extracts was accurately done on mettle AB54 balance.

500µl of test organisms freshly thawed stock was inoculated into 50ml of sterile middle brook 7H9/ADC broth medium and incubated at 30ºC for 5-7days. The optical density of resulting culture was measured using a UV/vis spectrophotometer. The optical density (OD) of resulting culture determined at 650nm was approximately 0.2 which equal to 10^9 cfu/ml.

III. RESULTS

Table 1: Extraction yield of Plant samples

Plant sample	Percentage Extraction yield(100g)
Waltheria indica	8.2
Ximenia americana	9.5
Pilostigma reticulatum	7.6
Striga hermonthica	9.4
Butyrospermum paradoxum	10.5

Cissampelos mucronata	10.3
Euphorbia hirta	11.6
Tamarindus indica	9.2
Erythrina senegalensis	8.5

Table 2: Preliminary Phytochemical Analysis of Plant Extracts

	EXTRACTS								
Parameter	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_8	E_9
Alkaloids	−	−	+	−	−	+	+	+	−
Tannins	−	+	+	+	+	+	+	−	+
Saponins	+	+	+	+	+	+	+	−	+
Flavonoids	−	−	+	+	+	−	+	−	+
Steroids	+	+	−	+	+	+	−	+	+
Terpenes	+	−	−	+	−	−	−	+	+
Glycosides	−	+	−	−	−	−	+	+	+
Anthraquinones	−	+	−	−	−	−	−	+	−
Phenols	+	−	−	−	+	−	+	−	−

Key: + = Present − = Absent

E1=Leaf extract of *Waltheria indica*, E2=Stem bark extract of *Ximenia americana*, E3=Leaf extract of *Pilostigma reticulatum* , E4=Aerial part extract of *Striga hermonthica* , E5=Leaf extract of *Butyrospermum paradoxum* , E6=Root extract of *Cissampelos mucronata*, E7=Whole plant extract of *Euphorbia hirta*, E8=Root-bark extract of *Tamarindus indica*, E9=Stem-bark extract of *Erythrina senegalensis*

Table 3: Preliminary Anti-mycobacterial Activity of Crude Plant Samples

Extracts/Doses(mg/ml)	*M. smegmatis*			*M. tuberculosis*		
	500	250	125	500	250	125
Waltheria indica	-	-	-	-	-	-
Ximenia Americana	-	-	-	-	-	-
Pilostigma reticulatum	-	-	-	-	-	-
Striga hermonthica	+	+	+	+	+	-
Butyospernum paradoxa	-	-	-	-	-	-
Cissampelos mucronata	+	-	-	+	-	-
Euphorbia hirta	+	+	-	+	+	-
Tamarindus indica	-	-	-	-	-	-
Erythrina senegalensis	+	+	+	+	-	-
**Control drug	+++	+++	+++	+++	+++	+++

Key: - = No activity + = Activity (inhibition of test organisms)
**Rifampicin=25µg/ml

IV. DISCUSSION

The results of the percentage extraction yield (table 1) of Leaf extract of *Waltheria indica*, Stem bark extract of *Ximenia americana*, Leaf extract of *Pilostigma reticulatum*, Aerial part extract of *Striga hermonthica*, Leaf extract of *Butyrospermum paradoxum*, Root extract of *Cissampelos mucronata*, Whole plant extract of *Euphorbia hirta*, Root-bark extract of *Tamarindus indica* and Stem-bark extract of *Erythrina senegalensis* was 8.2, 9.5, 7.6, 9.4, 10.5, 10.3, 11.6, 9.2 and 8.5 respectively. The result of the preliminary phytochemical screening of the methanol extracts of the plants was presented in table 2. The result revealed that the Leaves extract of *Waltheria indica* L. contained saponins, steroids,terpenes and phenols but showed a negative test for alkaloids, tannins, flavonoids, glycosides and anthraquinones. The leaves of *Ximenia americana L.* contained tannins, saponins, steroids, glycosides and anthraquinones but showed a negative test for alkaloids, flavonoids, terpenes and phenols. The Leaf extract of *Pilostigma reticulatum* (DC) Hochst contained alkaloids, tannins, saponins and flavonoids but showed a negative test for steroids, terpenes, glycosides, anthraquinones and phenols. The Root extract of *Cissampelos mucronata*. A. Rich contained alkaloids, tannins, saponins and steroids but showed a negative test for flavonoids, terpenes, glycosides, anthraquinones and phenols. The extract of Whole plant of *Euphorbia hirta L.* contained alkaloids, tannins, flavonoids, saponins, and glycosides but showed a negative test for steroids, terpenes and anthraquinones. *The root-bark of Tamarindus indica L.* contained alkaloids, steroids, terpenes, glycosides, and anthraquinones but showed a negative test for tannins, saponins, flavonoids and phenols. The stem-bark of *Erythrina senegalensis DC.* contained tannins, saponins, flavonoids, steroids, terpenes and glycosides but showed a negative test for alkaloids, anthraquinones and phenols. The aerial part of *Striga hermonthica* (Del.) Benth contained tannins, saponins, flavonoids, steroids and terpenes but showed a negative test for alkaloids, glycosides, anthraquinones and phenols. The leaf extract of *Butyrospermum paradoxum* (Gaertn.f.)Heppe contained tannins, saponins, flavonoids, steroids and phenols but showed a negative test for alkaloids, terpenes, glycosides and anthraquinones. The presence of these chemical constituents make the plants useful for treating several ailments and the curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites. The result of the preliminary anti-mycobacterial screening of the plants crude extracts and the standard antibiotic drug (25µg/ml) against the tested organism in well diffusion is presented in table 3. The result revealed that only four (4) plants out of the nine (9) selected plants exhibited anti-mycobacterial activity (500 - 125mg/ml) when tested for anti-mycobacterial activity (Table 3). The crude extract of the aerial parts of *S. hermonthica* inhibited growth of the tested organisms at 500mg/ml but at lower concentrations (125mg/ml) the extract was not active against *M. tuberculosis*. The crude extract of stem bark of *E. senegalensis* was also active at 500mg/ml against the tested organisms but did not demonstrated activity against *M. tuberculosis* at lower concentrations. The root of extract of *C. mucronata* was only active at 500mg/ml but was inactive at lower concentrations and the whole plant extract of *E. hirta* was only active at concentrations per well of 500-250mg/ml against both *M. smegmatis* and *M. tuberculosis*. No activity was observed for the other five (5) plants extract used in the study that is *W. indica, X. americana, P. reticulatum, B. paradoxa* and *T. indica. S. hermonthica* has been used extensively in folkloric medicine in many parts of Africa (Choudhury *et al*., 1998; Atawodi *et al*., 2003). It has a wide range of medicinal uses which include abortificient effect, dermatosis, leprosy, ulcer, pneumonia and jaundice remedy, trypanocidal effects, antibacterial and anti-plasmoidal activities (Choudhury *et al*., 1998; Okpako and Ajaiyeoba, 2004). *Piliostigma thonningii (Caesalpiniaceae*) is frequently used for treating chest pain, hemoptysis and sore throat. Similarly, decoction of the fresh young leaves, dried stem bark and root bark were earlier reported to have been used for the treatment of chest complaint, cough, fever and expectorant (Gill, 1992). *Tamarindus indica L.* is given as a remedy for indigestion and colic. It is also used in the treatment of hemorrhoid, asthma, gingivitis and eye inflammation. An infusion of the roots has curative value in chest complaints and as ingredient in management of leprosy (Julia and Miami 1987). *Waltheria indica* is used in Nigeria for the treatment of diarrhoea, infertility, skin diseases, gonorrhea and for relieving pains (Zailani *et al*, 2010). The whole plant is locally used to treat cough, haemorrhage, fever, and malaria (Diallo *et al*., 1999). *X.americana L.* is used to treat skin problems, headaches, leprosy, heart complaints, haemorrhoids, sexually transmitted disease, guinea worm, sleeping sickness, and oedema, fever, colds, mouthaches and as a laxative (Abdalfatah *et al*., 2013). *Cissampelos mucronata* is popular among traditional healers in Nigeria in treatment of anti-diarrhoeal conditions. Traditionally the root bark is used to relieve dysmenorrhoea, to prevent abortion and also as a sedative (Ogwal *et al*., 1996) Antispasmodic activity (Offiah *et al*., 2003) and anti-ulcer activity (Akah and Nwafor 1999). *Butyrospermum paradoxum* (Gaertn.f.) Hepper is used for treating asthma, expectorant, tuberculosis and whooping cough. The decoction of seeds for treating catarrh condition and nasal decongestion (Gill, 1992). The stem bark of *Erythrina senegalensis* has shown strong analgesic and anti-inflammatory effects (Saidu *et al*., 2000), and Udem *et al*. 2010 have reported the low toxicity of stem bark extract of *Erythrina senegalensis* DC. *Euphorbia hirta* is used as ear drops and in the treatment of boils, sore and wound healing (Ogueke *et al.*, 2007). The plant has been used in treating respiratory ailments, especially cough, coryza, bronchitis and asthma. In India it is used to treat worm infestations in children and for dysentery, gonorrhoea, jaundice, pimples, digestive problems and tumours (Sandeep *et al*., 2009).

In conclusion, the global threat of tuberculosis required new anti-mycobacterial agents with low toxicity and improved mode of action in order to reduce the prevalence of multi-drug resistant mycobacterial strains. Therefore, these findings have provided some prerequisites results for further studies on the vivo activity and toxicity of the active plants in the study.

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